interpretation of the usually complex diffusion measurements obtained in living cells.

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Study of the behavior of triacylglycerols in membrane bilayers and the formation of lipid droplets using all-atom molecular dynamics simulations

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Triglycerides, or triacylglycerols (TAGs), are neutral lipids consisting of a glycerol head group and three fatty acid chains. They serve as the main component of animal fat and are a primary energy store for humans. An excess of TAGs in the human body, a condition known as hypertriglyceridemia, can be an indicator for weight-related disease. TAGs display unique properties when concentrated in lipid bilayers, forming aggregates in the hydrophobic region. This lends itself to TAG's significant role in the genesis and constituency of lipid droplets in the endoplasmic reticulum (ER). Additionally, the formation of these blister-like clusters in membrane bilayers are particularly prominent in malignant cells. Understanding the structure and dynamics of TAG aggregates could provide new insights into the treatment of cancer, as well as diseases related to hypertriglyceridemia. Using all-atom simulations of varying TAG percentages within ER or POPC systems, this study exhibits the conditions in which TAG aggregation could feasibly occur, which practically guides how to build a TAG-containing system using CHARMM-GUI Membrane Builder.

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Insights of phase separation in bilayers of ternary mixtures with different sterols

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It has been shown that polyene antimycotics are selective according to the sterol present in the membrane, leading to stronger activity in fungal cells. However, it is not clear the role of the membrane structure in ionophoretic activity. In recent years, there has been a debate about if the homogenous model of the membrane is accurate or there exist nanoscopic domains enriched with sphingomyelin and cholesterol. The influence of sterol and its interaction with sphingomyelin can provide information on ordered domain formation. The purpose of this study is to compare the effect of cholesterol or ergosterol upon domain formation in mixtures of POPC/PSM with different sterol concentrations. We performed microsecond timescale all-atom molecular dynamics simulations, using GROMACS and the Slipids force field, for this system with 0 and 20 mol% of either cholesterol or ergosterol. The evaluated properties are lipid order parameters, membrane thickness, lipid local enrichment, and radial distribution functions. Results show a drastic increase in chain ordering effect on the POPC tail chains in the ternary POPC/PSM/sterol bilayer, which is not sterol dependent. The order parameters of PSM tail chains in the ternary POPC/PSM/sterol lipid bilayer are close to the original PSM values, and it seems sterol independent. The enrichment analysis shows cholesterol preference to associate with POPC over PSM, while the opposite occurs in ergosterol system. Finally, we compare our thickness results with AFM, where the difference between cholesterol and ergosterol is observed. We believe structural and conformation differences could explain the influence of sterol on biomolecular processes related to polyene ionophoretic activity.

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Quantifying cellular mechanics with image analysis and mathematical modeling

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Cells are highly dynamic, changing shape through stretching or contracting, moving and dividing. Recent advances in computational image analysis allow for quantification of cellular dynamics, but there does not exist a robust and consistent process. The purpose of our work was to create a process with image analysis software to measure changes in cell area and perimeter over time, then create a mathematical model to predict the amount of tension present in the cell membrane. Our work uses ImageJ, with a machine learning plugin, WEKA to identify and quantify cell membranes from experimental time lapses. We developed a process to optimize the preprocessing of the experimental time lapses then defined best practices for training WEKA through identifying the cell membrane, cell interior, and background. After WEKA identified cell membranes, we thresholded the images to only highlight cell membranes, and measured each identified cell. We validated our results with the dice similarity coefficient by comparing WEKA identified membranes to manually identified membranes. To predict tensions in the cell membrane, we created a mathematical model in MATLAB that used the measured cell perimeters to calculate the likely tension present in the membrane if the cell membrane behaved like a Hookean spring. From the experimental data we calculated the strain rate, the ratio of the change in length to original length, and tension by multiplying the change in length by a spring constant. The process we developed is mostly automated which means we can quantitatively describe a cell or culture of cells faster and with more accuracy than prior manual "by eye" calculations. We also have the capacity to identify and quantify large clusters of cells which allows for determining statistical significance between different control or experimental conditions.

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Diffusion study of hetero-oligomers detected by fluorescence cross-correlation spectroscopy (FCCS)

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The dynamics of lipids and proteins are crucial in the mechanisms of cellular functions. The robustness of fluorescence correlation spectroscopy (FCS) makes it possible to quantify the diffusion and molecular interactions at nanomolar concentration in biological systems. We developed novel, economical methods with the use of super-continuum laser and spectral deconvolution with experiment-specific excitation wavelengths and emission spectra for revealing the interactions between up to four spectrally overlapping fluorophores. We applied our method to perform fluorescence cross-correlation spectroscopy (FCCS) and demonstrate the induction of correlated diffusion of lipid vesicles and polystyrene nanoparticles. We select the specific laser wavelengths from the fiber laser to excite the diffusers within the diffraction-limited spot. The fluorescence emission passes through a cleanup filter and a prism prior to being collected by a sCMOS or EMCCD camera at 10 kHz. The intensity versus time of each color channel is extracted through a nonlinear least-square fitting of each camera frame and temporally correlated via custom software. From the auto- and cross-correlation functions, we measure the diffusion rates and binding partners of the molecules. We have measured the induction of aggregation of nanobeads and large unilamellar vesicles (LUVs) in solution upon the addition of PBS and BSA, respectively. Ongoing experiments examine the molecular mechanism of lipolysis by measuring the correlated diffusion on lipid droplets. Lipolysis associated proteins such as alpha beta hydrolase domain containing protein 5 (ABHD5), perilipin (PLIN) and adipose triglyceride lipase (ATGL) undergo homo- and hetero-oligomerization upon addition of lipolysis-stimulating ligands. These studies will provide further understanding of how lipolysis is regulated via protein interactions with the lipid droplet membrane.

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Comparative molecular dynamics simulation studies of realistic eukaryotic, prokaryotic, and archaeal membranes

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The OPM (Orientation of Proteins in Membranes) database currently holds, classifies, and annotates around 14,000 three-dimensional structures of transmembrane and peripheral membrane proteins and peptides positioned with respect to membrane boundaries. Developing uniform templates of biological membranes with realistic native-like lipid composition would provide a convenient and accurate platform for researchers who simulate membrane-associated proteins and peptides in natural environments. In this study, for the first time, we performed the standardized molecular dynamic simulations and comparative analysis of structures and properties of 18 native-like membrane models. Multi-component lipid bilayer systems with lipid composition of different eukaryotic, prokaryotic to archaeal membranes, were modeled using CHARMM-GUI Membrane Builder and simulated with the CHARMM36 force field and OpenMM simulation package with all explicit atoms. Each system contained more than 200 lipid molecules representing from 4 to 23 lipid types. We analyzed the structural properties of these systems, including membrane thicknesses, membrane area compressibility moduli, deuterium order parameters, sterol tilt angles, mass density profiles, and areas per lipid. The obtained structures of 18 membrane systems can be used as templates for the future all-atom molecular dynamics simulations of realistic biological membranes containing proteins in the native-like lipid environment. To

facilitate these simulations, the CHARMM-GUI Membrane Builder will be directly connected to the OPM database.

341-Pos
Visualizing dynamic membrane encounters with a DNA zipper probe
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The cell membrane is a complex structure composed of a diverse array of lipids and proteins that transiently interact to maintain its lateral organization and function. Preferential interactions among certain lipids like cholesterols can result in the formation of membrane sub-compartments, such as lipid domains, which will act as platforms for more specific cell signaling. These membrane lipid interactions are fast and therefore have not been well characterized or visualized with traditional analytical or imaging techniques. Herein, we designed fluorescent cholesterol-DNA probes, named as ''DNA Zipper'', which can spontaneously modify onto cell membranes and be used for reporting membrane transient interactions and lipid domain formations. The ''DNA Zipper'' probes work by stabilizing short-lived cholesterol interactions via variable DNA hybridizations, in a way just enough for them to be visualized without being dominated by DNA interactions. By quantifying the Förster resonance energy transfer (FRET) efficiency occurring between a donor and acceptor cholesterol-DNA probe, we successfully explored the impacts of various membrane lipid-depletion or lipid-enrichment environments on the lipid domain formations on live-cell membranes. Furthermore, we investigated the role of the formation of stabilized lipid domains at the onset of T-cell activation. Interestingly, the activation of T-cell receptor signaling is spatiotemporally highly correlated with the level of DNA Zipper signals and the formation of lipid domains. We believe these DNA-based probes will be an important analytic tool that can be broadly used to image and study membrane heterogeneous structures and interactions.

342-Pos
Polarized deformations: response of giant unilamellar vesicles to osmotic deflation under confinement
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Membranes are dynamic features of living cells that undergo continuous changes to facilitate many cellular functions including but not limited to motility, cell division, and phagocytosis. Although many of these processes are protein-assisted, functionality can also emerge through simple physical stresses and perturbations coupled with ability of the cellular membrane to regulate their area. This remarkable property of surface area homeostasis, which buffers membrane tension, is often achieved through strikingly simple and chemically non-specific physical-processes such as (1) folding, which traffics molecules with the contiguous end-membrane system; (2) endocytosis, which lowers the surface area; and (3) exocytosis, which expands it. With membrane being at the forefront of such perturbations, it becomes crucial to understand how membranes respond to physical stresses. Stress response of membranes has been widely studied using cell mimics such as giant unilamellar vesicles (GUVs). In most of these cases, the perturbation is isotropically applied, which rarely is the case of the native cell environment. We observe that when a degree of stress anisotropy is introduced (facilitated by weak adhesion of GUV basal region to the substrate in our case) coupled along with a global osmotic deflation, the vesicle undergoes rampant inward multivesiculations in conformity with tensional homeostasis. This cluster of microscopic invaginations is spherical and generally 3-4 um in radius. This is structurally distinct from free GUV deformations on deflation. Strikingly, even though these invaginations germinate in the basal planes, the accompanying density contrast drives them to accumulate at the distal surface producing a global morphological polarity reminiscent of many adherent eukaryotic cells.

343-Pos
Lipids in channel pore: potential gating mechanism or simulation artifact?
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Coarse-grained (CG) Martini force field in molecular dynamics simulations is currently the most popular model to study protein-lipid interactions for large protein size and long-timescale. Literature has reported lipids entering the ion channel pore during CG simulation, such as in mechanosensitive Piezo1 channel1, however, no experimental evidence addressed whether these lipids should be in the pore. Moreover, our previous 7.9μs simulation of a reduced Piezo1 all-atom simulation did not show permanent pore occlusion by lipids in the nonconducting state. The discrepancy of lipid distribution in the pore from CG model with all-atom (AA) model simulation triggered us to investigate whether these lipids present in the pore are potential gating mechanism or an artifact event from CG simulation due to simulation protocol or force field. We constructed a pore model of Piezo1 channel consisting only the cap, pore and CTD domains (V1976 to R2546), tested several different equilibrium protocols in both CG and AA simulations and calculated absolute binding free energy of lipids in Piezo1 pore. Results show in CG models using lipid headgroup-only restraint led to trapped lipids in the Piezo1 upper vestibule but not in AA models, and this can be avoided by using whole lipid restraint for CG models in equilibrium stage. It indicated that initial solvation algorithm and the type of lipid restraint during equilibrium in CG and AA simulation can result in a drastically different hydration pattern in pore and led to trap lipids in pore in CG model. $+41.0 \pm 0.2$ kcal/mol of absolute binding free energy of lipids in the pore vs. in bilayer suggested moving lipids from bilayer membrane to the upper vestibule is not favorable. To avoid such artifacts, one could be never too careful when setting up CG simulations for equilibrium.

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Colony-like protocell superstructures
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We report the formation, growth, and dynamics of model protocell superstructures on solid surfaces, resembling single cell colonies. These structures, consisting of several layers of lipidic compartments enveloped in a dome-shaped outer lipid bilayer, emerged as a result of spontaneous shape transformation of lipid agglomerates deposited on thin film aluminum surfaces. Collective protocell structures were observed to be mechanically more stable compared to isolated spherical compartments. We show that the model colonies encapsulate DNA and accommodate non-enzymatic, strand displacement DNA reactions. The membrane envelope is able to disassemble and expose individual daughter protocells, which can migrate and attach via nano-tethers to distant surface locations, while maintaining their encapsulated contents. Some colonies feature 'exo-compartments', which spontaneously extend out of the enveloping bilayer, internalize DNA, and merge again with the superstructure. A continuum elastohydrodynamic theory that we developed reveals that the subcompartment formation must be governed by attractive van der Waals (vdW) interactions between the membrane and surface. The balance between membrane bending and vdW interactions yields a critical length scale of 273 nm, above which the membrane invaginations can form subcompartments. The findings support our hypotheses that in extension of the 'lipid world hypothesis', protocells may have existed in the form of colonies, potentially benefiting from the increased mechanical stability provided by a superstructure.

345-Pos
Membranes and invisibility cloaks
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Pioneering theoretical works of transformation optics and conformal mapping have scientifically scrutinized existence of invisibility cloaks. Inspirational theoretical works of Pendry and Leonhardt significantly amplified and accelerated search of metamaterials acting as invisibility cloak. Consequently, invisibility cloaks at micro, optical and visible frequencies have been found. However, despite of progress theoretical works also indicated that invisibility cloaks retain size dependence dictated by incident wavelength and for visible light must remain tiny. In this conference proceeding we intend to ask if a membrane might act as invisibility cloak for cell. Here, we do not follow Pendry and Leonhardt original ideas. Instead, we study existence of membranes acting as invisibility cloaks by recently derived equations of motions for moving manifolds in electromagnetic fields (Svintradze 2017-2020). If initial field lines and distorted field lines coincide then the object is cloaked against electromagnetic waves. This can be achieved by two different ways: 1) according to conformal mapping and optical transformations or 2) by moving shapes. While optical transformation and conformal mapping depend on magnetic permeability and electric permittivity transformations, are only applicable to not moving shapes and are limited to certain sizes, our approach is free from permittivity and permeability and any object of any size can be cloaked if covered by properly moving hyper surface. Therefore, if artificial membrane is found that satisfies solutions to our equations of motion (Svintradze 2017-2020) then the membrane would act as invisibility cloak for the cell it encloses and it will not be restrained by the size. Using the equations of motions, we can argue, that any system bounded by the moving surface, can be hidden from an electromagnetic field only if the surface dynamics satisfies specific solutions to our